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Prospective study of antibody to human papilloma virus type 16 and risk of cervical, endometrial, and ovarian cancers (United States)

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Abstract

Objective: Human papilloma virus (HPV) is frequently detectable in cancers of the cervix, vagina, and vulva, but its role in endometrial and ovarian cancers is less certain. This analysis aimed to examine the association of presence of HPV type 16 (HPV-16) antibodies with subsequent risk of cervical, endometrial, and ovarian cancers.
Methods: In a prospective study enrolling over 15,000 pregnant women, pre-cancer sera from women who developed cervical (n = 83), endometrial (n = 34), and ovarian (n = 35) cancers were compared with sera from 172 control women frequency-matched by age group and race.
Results: HPV-16 seropositivity (OR = 2.0, 95% CI 1.0–3.4) was associated with cervical cancer, with the association more prominent for cancers occurring within 10 years of serum sampling (OR = 2.3, 95% CI 1.0–5.3) than cancers occurring later (OR = 1.6, 95% CI 0.75–3.6). Overall, the associations between HPV-16 seropositivity and endometrial (OR = 1.6, 95% CI 0.64–3.8) and ovarian cancers (OR = 1.1, 95% CI 0.43–2.8) were not significant, although the odds ratios for those cancers occurring within 20 years after serum sampling were similar to that for cervical cancer (OR = 2.2 for both).
Conclusions: Our results confirm that HPV-16 infection precedes the development of cervical cancer. Predictability of HPV-16 seropositivity for risk of other female cancers warrants further investigation.

Introduction

Infection with specific types of human papillomavirus (HPV) is thought to be a causal factor for cervical cancer [1–4] and its precursor, cervical interepithelial neoplasia (CIN) grade III [5, 6]. Experimental studies have shown that HPV transforms and immortalizes cervical epithelial cells *in vitro*, and that HPV E6/E7 oncogenes are selectively retained and expressed in cancer cells [1]. Almost all of the cervical cancer specimens contain HPV DNA when tested with sensitive hybridization methods [2]. HPV-16 is the most common HPV type in squamous cell cervical carcinoma, accounting for approximately 50% of HPV DNA positive tumors in the United States [1].

HPV-16 viruslike particles (VLPs) expressing HPV-16 late proteins L1 and L2 have been derived from a variety of systems including *E. coli*, yeast, and recombinant baculovirus and vaccinia virus systems [7]. Antibodies to these HPV-16 VLPs have been shown to correlate with the presence of HPV-16 DNA in tumors [7, 8]. Antibody assays based on these HPV-16 VLPs have good to moderate specificity and interlaboratory agreement [9].

Evidence linking HPV-16 seropositivity and cervical cancer comes primarily from case–control studies, in which the presence of various HPV antibodies was determined in subjects whose cancer had already been diagnosed [10–14]. However, to demonstrate that HPV infection precedes the development of cancer, it is important to examine the association of cancer risk with

HPV-16 antibody prior to diagnosis, which has been done in only a few prospective studies [8, 14–17].

HPV may also be detected in other cancers of the female lower genital tract, including cancers of the vulva, vagina, and perineum [14, 18, 19], but its role in the development of cancers of the upper genital tract, such as cancers of the endometrium and ovary, is less clear. Evidence that supports the association of HPV infection with ovarian cancer includes the observation that HPV binds and immortalizes non-squamous epithelial cells, such as those of the ovary [20, 21]. However, some studies have found HPV DNA in ovarian tumor cells [22, 23], while others have not [24]. Studies of HPV DNA in endometrial cancer also show inconsistent results [23–25]. Limited serological data from a cross-sectional study did not support an association of HPV infection with cancers of these sites [26]. To date, the association of HPV-16 serostatus with risks of endometrial and ovarian cancers has not been evaluated in prospective studies.

To address these issues we compared the prevalence of HPV-16 antibody in women who subsequently developed cervical, endometrial, or ovarian cancers and those free of cancer in a large prospective study.

Subjects and methods

Study population

The prospective Child Health and Development Study was originally established in 1959 to study the association of biologic, genetic, medical, and environmental factors during pregnancy with the development of the offspring [27]. A total of 15,528 women who were members of the Kaiser Foundation Health Plan and resided in the Oakland, California area were enrolled in the cohort during their pregnancies between June 1959 and September 1966. Baseline questionnaires collected medical and demographic data as well as reproductive history from all women. In-depth interviews collected detailed information regarding health-related matters, including smoking habits and alcohol consumption. Serum samples were collected at initial enrollment and stored at −20 °C until use. The follow-up study was reviewed and approved by the Institutional Review Boards of the University of California at Berkeley, the Western Consortium for Public Health, and the Public Health Institute (Berkeley, CA).

Case ascertainment and control selection

Cancer cases occurring in cohort members were identified through linkage with the California Cancer Registry or death certificates in California through October 1993. Cancer reporting to the registry was estimated to have been 97% and 75% complete, statewide, through 1991 and 1992, respectively. Cancer sites of interest were categorized as the following according to ICD for Oncology, 2nd edition (ICD-0-2; 1990): cervix uteri (C53.0, C53.1, C53.8, C53.9); endometrium or corpus uteri (C54.1 and C54.9); and ovary (C56.9). Cancer diagnoses were microscopically confirmed for 173 of the 174 cancer cases identified. Ninety-nine cervical cancers (47 *in situ*, 52 invasive), 39 endometrial cancers, and 36 ovarian cancers were identified. A total of 194 control subjects were selected among women in the same study cohort who had not been reported to have any cancers through October 1993, frequency-matched to the combined cancer cases by decade of birth and race.

Laboratory methods

Presence of antibody to HPV-16 in stored serum samples, collected at time of enrollment, was determined using an enzyme immunosorbent assay (ELISA) as previously described [7, 28]. VLP antigen for this assay was produced from Sf9 insect cells infected with a recombinant baculovirus expressing the L1 and L2 proteins of HPV-16. Serum samples were tested at 1:20 dilution in duplicate. Optical density (OD) at 405 nm was determined in an automated ELISA plate reader (Bio-Tek, Winooski, VT) and adjusted to the values for two control specimens with different reactivity (high and low) on each plate [9, 28]. All OD values were determined by laboratory personnel who were blinded to the subjects' case–control status.

Statistical analyses

Based on the cutoff determined and validated in previous investigations, averages of the duplicate adjusted OD values were classified into four levels: "negative" (−) if the OD value was <0.904, "indeterminate" if $0.904 \le OD < 1.017$, "low positive" if $1.017 \le OD < 1.327$, and "high positive" if $OD \ge 1.327$ [9]. Seropositivity for HPV-16 in some analyses was examined as a dichotomous variable as "positive" ($OD \ge 1.017$) *versus* "negative" ($OD < 0.904$), excluding indeterminate OD values. Age at serum sampling was categorized as <20, 20–24, 25–29, 30–34, and ≥ 35 years old. Smoking status was dichotomized as ever *versus* never as of the time of serum sampling. As a proxy of socioeconomic status we used the subject's educational level at enrollment as a categorical variable (did not complete high school, high school graduate, high school plus some college, college graduate). Age at menarche was also examined as an ordered categorical variable at approximate quartiles (≤11, 12, 13, and ≥14 years old). Years between blood sampling and cancer diagnosis was dichotomized at 10 years for cervical cancers and at 20 years for endometrial and ovarian cancers (early *versus* late onset). Self-reported race/ ethnicity was categorized as White, Black, Hispanic, Asian, and other.

Chi-square statistics were used to test the significance of the associations among variables as well as to compare characteristics between each cancer group and the combined control group. Cancers of early *versus* late onset were each compared against the control groups. Relative risk of each cancer was estimated by the odds ratio (OR) derived from multiple logistic regression analysis [29]. A test for trend for the association between antibody level and cervical cancer risk was carried out by the likelihood ratio test after including HPV-16 as a categorical (four categories) variable in the logistic regression model. The Wald-type 95% confidence interval (CI) was calculated. Statistical tests were two-sided. Because there were only a few cancer cases among Hispanics and Asians, these ethnic groups were excluded from the logistic regression analyses.

Results

Overall, 28 (29%) of the 99 cervical cancer patients, 11 (29%) of the 39 endometrial cancer patients, eight (22%) of the 36 ovarian cancer patients, and 43 (22%) of the 194 control subjects were HPV-16 antibody positive at study enrollment (Table 1). Median years between serum sampling and cancer diagnosis were 10 years for cervical cancer and over 20 years for both endometrial and ovarian cancers, respectively. Smoking status (OR = 0.6, 95% CI 0.3–1.0) and educational level (OR = 0.8, 95% CI 0.6–1.1) were each inversely associated with HPV-16 seropositivity in this study cohort. After excluding Hispanic and Asian subjects, a total of 83 subjects with cervical cancer, 34 with endometrial cancer, 35 with ovarian cancer, and 172 control subjects were included in the logistic regression analysis. With adjustment for age at serum sampling and race, HPV-16 seropositivity (OR = 2.0, 95% CI 1.0–3.4) was associated with an increased risk of subsequent cervical cancer (Table 2). A significant association was also found between antibody level and risk of cervical cancer (p for trend = 0.01). With adjustment for age group at serum sampling and race, smoking (OR = 1.7, 95% CI 0.9–3.3) and age at menarche (OR = 0.8, 95% CI 0.6–1.1) were not an independent risk factor for cervical cancer in our series. Adjustment for these variables did not affect the ORs for HPV-16 antibody positivity and risk of cervical cancer. In contrast, a lower educational level was strongly associated with an increased risk of cervical cancer (OR = 0.5, 95% CI 0.3–0.7). The OR for the association of HPV-16 seropositivity and risk of cervical cancer was slightly reduced (OR = 1.7; 95% CI 0.8–3.4) after additional adjustment for educational level.

HPV-16 positivity was a significant predictor for cervical cancers within 10 years of serum sampling (OR = 2.3, 95% CI 1.0–5.3) but not for those that occurred later (OR = 1.6, 95% CI 0.8–3.6). In addition, HPV-16 antibody positivity was significantly associated with risk of invasive cervical cancer (OR = 2.4, 95% CI 1.1–5.1) but the association was weaker and not significant for *in-situ* carcinomas (OR = 1.5, 95% CI 0.7–3.6). With adjustment for age at serum sampling, race, and HPV-16 antibody positivity, smoking was independently associated with diagnosis of *in-situ* carcinomas (OR = 3.4, 95% CI 1.3–8.7) but not with that of invasive cancers (OR = 1.6, 95% CI 0.7–3.8).

The associations of HPV antibody with risk of endometrial and ovarian cancers were similarly analyzed. Overall, HPV-16 antibody positivity was not significantly associated with endometrial cancer (OR = 1.6, 95% CI 0.6–3.8) or ovarian cancer (OR = 1.1, 95% CI 0.4–2.8) (Table 2). With adjustment for age at serum sampling and race, smoking (OR = 1.6; 95% CI 0.7–3.9), educational level (OR = 0.7; 95% CI 0.4–1.1), and age at menarche (OR = 0.6; 95% CI 0.3–1.1) were not significant risk factors for risk of endometrial cancer. Similarly, neither smoking (OR = 0.8, 95% CI 0.3–1.9) nor educational level (OR = 0.8, 95% CI 0.5–1.2) was significantly associated with risk of ovarian cancer. Age at menarche, however, was associated with risk of ovarian cancer (OR = 1.6 per year, 95% CI 1.1–2.3). The OR for HPV-16 antibody positivity was elevated, albeit not significantly, for endometrial cancers occurring within 20 years of serum sampling (OR = 2.2, 95% CI 0.6–8.3), while no association was found for those occurring later (OR = 1.1, 95% CI 0.3–3.6) (Table 2). HPV-16 antibody positivity was more frequent for ovarian cancers occurring within 20 years of serum sampling (OR = 2.2, 95% CI 0.6–8.1) but not for those occurring later (OR = 0.6, 95% CI 0.2–2.3).

Discussion

HPV infection is causally associated with the occurrence of cervical cancer, the second most common malignancy among women worldwide [30]. While less sensitive than molecular-based methods such as polymerase chain reaction, serologic assays are often the only tool

Table 1. Characteristics of study subjects in the Child Health and Development Study (1959–1993) by cancer site[a]

	Cancer cases			Controls (n = 194)
	Cervical (n = 99)	Endometrial (n = 39)	Ovarian (n = 36)	
Age at serum sampling (years)				
<20	15 (15%)	2 (5%)	2 (6%)	18 (9%)
20–24	31 (32%)	5 (13%)	5 (14%)	55 (28%)
25–29	24 (24%)	10 (26%)	8 (22%)	44 (23%)
30–34	16 (16%)	8 (20%)	7 (19%)	39 (20%)
35+	13 (13%)	14 (36%)	14 (39%)	38 (20%)
Race/ethnicity				
White	49 (50%)	31 (79%)	31 (86%)	121 (62%)
Black	34 (34%)	3 (8%)	4 (11%)	51 (26%)
Asian[b]	6 (6%)	3 (8%)	1 (3%)	9 (5%)
Hispanic[b]	5 (5%)	2 (5%)	0 (0%)	7 (4%)
Other[b]	5 (5%)	0 (0%)	0 (0%)	5 (3%)
Smoking status				
Ever	53 (65%)	22 (63%)	14 (47%)	93 (53%)
Never	28 (35%)	13 (37%)	16 (53%)	83 (47%)
Highest educational level				
Less than high school	32 (40%)	5 (14%)	2 (7%)	26 (16%)
High school	30 (37%)	13 (37%)	16 (52%)	70 (40%)
Some college	18 (22%)	12 (35%)	7 (22%)	43 (24%)
College and above	1 (1%)	5 (14%)	6 (19%)	35 (20%)
Age at menarche (years)				
≤11	13 (25%)	3 (19%)	1 (5%)	26 (20%)
12	13 (25%)	4 (25%)	5 (26%)	39 (29%)
13	14 (28%)	7 (44%)	8 (42%)	42 (31%)
≥14	11 (22%)	2 (12%)	5 (26%)	26 (20%)
Years from serum sampling to cancer diagnosis				
<10	42 (43%)	3 (8%)	4 (11%)	N/A
10–19	31 (31%)	11 (28%)	9 (25%)	N/A
20–29	19 (19%)	26 (61%)	18 (50%)	N/A
≥30	7 (7%)	1 (3%)	5 (14%)	N/A
HPV-16 antibody				
OD < 0.904	59 (60%)	27 (69%)	26 (72%)	133 (69%)
0.904 ≤ OD < 1.017	11 (11%)	1 (3%)	2 (6%)	17 (9%)
1.017 ≤ OD < 1.327	11 (11%)	8 (21%)	3 (8%)	26 (13%)
1.327 ≤ OD	17 (18%)	3 (8%)	5 (14%)	17 (9%)

N/A, not applicable.
[a] Due to missing values the numbers do not always sum to the total.
[b] Excluded from the analysis.

available to measure HPV infection in large population-based epidemiologic studies. In the present study we used HPV-16 VLPs antibody as a marker of exposure to HPV-16 [31], the HPV type most frequently detected in cervical cancer. A significant two-fold increase in cervical cancer risk among women who had been HPV-positive prior to diagnosis is consistent with the notion that HPV infection is a risk factor for subsequent development of cervical cancer.

The association of antibodies to various HPV-16 epitopes and the risk of cervical cancer has been examined in several other prospective studies, which generally found a higher OR, ranging from 2.4 to 7.5 [14–16]. A lower OR in the present study appears to be, at least in part, due to a higher HPV seroprevalence among control subjects (22%) compared to that reported in other studies (<10%), including two studies from our group [26, 32]. Because the cancers had occurred up to 32 years after blood collection in the present study as compared to a less than 15-year interval for other studies, a number of our cases and controls may have been infected with HPV-16 after blood samples had

Table 2. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for risk of cervical, endometrial, and ovarian cancers by the presence of HPV-16 antibody within specified time intervals between serum sampling and diagnosis (early *vs.* late) in the Child Health and Development Study[a]

	Cancer sites					
	Cervix		Endometrium		Ovary	
	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
Overall	74	2.0 (1.0–3.4)	33	1.6 (0.6–3.8)	34	1.1 (0.4–2.8)
Early[b]	33	2.3 (1.0–5.3)	14	2.2 (0.6–8.3)	12	2.2 (0.6–8.1)
Late	41	1.6 (0.8–3.6)	19	1.1 (0.3–3.6)	22	0.6 (0.2–2.3)

[a] The models adjust for age at serum sampling and race. Excludes subjects with indeterminate OD values.
[b] Early cancers are those occurring <10 years for cervical cancers and <20 years for endometrial and ovarian cancers. Some cancer cases and controls were excluded from the multivariate analysis because of missing data on covariates.

been taken. However, this mechanism does not fully explain our lower OR, since the observed association for cancers occurring within 10 years of serum sampling was still relatively low (OR = 2.3). Although missed cancer diagnosis among those who moved out of the area could have contributed to a lower OR, this is unlikely to have caused a major effect in our study, unless emigration was associated with HPV seropositivity. Differences in quality of stored sera, serologic test performance, population characteristics such as baseline frequency of infection with HPV-16, infection with crossreactive HPV types (31, 33, 35 and 58) [33], and chance may have attenuated the association in the present study. A recent study has reported that antibodies to hepatitis C virus were stable in serum stored at −20 °C for nearly 50 years [34]. It therefore seems unlikely that the detection of HPV-16 antibodies in the present study was hampered by the storage conditions of these sera.

The association of HPV antibody positivity and cervical cancer risk was slightly stronger for invasive cancers as compared to *in-situ* cancers (OR = 2.4 *vs.* 1.5). Although the time interval from serum sampling to cancer diagnosis was similar for invasive and *in-situ* cancers (data not shown), individuals who became infected with HPV-16 after blood collection may have a greater opportunity to develop *in-situ* cancers than invasive cancers. Thus, a weaker association of HPV antibody positivity with *in-situ* cancer compared to invasive cancer may be partly explained by misclassification of HPV antibody status among patients with *in-situ* cancers.

The role of smoking in the development of HPV-associated cervical cancers has been suggested in many studies [35–40]. However, the effect of smoking independent of the major risk factor, HPV infection, remains unclear [6, 41]. In the present study the association of smoking and risk of cervical cancer diagnosis was found only for *in-situ* carcinoma. Whether there is a biological explanation for this observation is at present uncertain. The association of HPV antibody positivity and risk of cervical cancer was stronger for cases diagnosed within 10 years of blood sampling compared to those diagnosed more than 10 years later (OR = 2.3 *vs.* 1.6). The finding raises the possibility that some of the subjects with longer intervals to cancer may have acquired HPV positivity after their bloods were drawn, or the expression of HPV infection increased over time.

Since HPV-16 seropositivity was inversely associated with the subject's education level, HPV infection may explain a part of the known association of socioeconomic status with cervical cancer. The association of education level with risk of cervical cancer in the present analysis was highly significant (OR = 0.5 per category), after adjusting for the HPV-16 seropositivity, age at serum sampling, race, and smoking status in the logistic regression model. Age at menarche, which has been associated with HPV-associated anal carcinoma [42], was not a risk factor for cervical cancers in the present study.

The present study is the first prospective analysis to evaluate the association of HPV-16 seropositivity with subsequent risk of endometrial and ovarian cancers. A non-significant two-fold increase in risk for these cancers occurring within 20 years of serum sampling in the present study warrants further investigation of the potential role of HPV-16 for cancers of non-epithelial sites. While random variation in the ORs in stratified analysis may have led to a spurious association, the strength of association (OR ~2) for these cancers is strikingly similar to that for a well-established association of HPV-16 with cervical cancer. However, since HPV DNA is not consistently found in the endometrial and ovarian tumor tissue, an alternative mechanism of carcinogenesis needs to be hypothesized before an etiologic association can be made. Furthermore, since our power to detect an association may have been limited by a relatively small number of cancer cases, a larger series and concurrent measurements of other molecular markers are desirable in future studies. Also, prospective follow-up of changes in the antibody level over time in serial, precancer blood samples would be useful. Recent observations that HPV-16 seropositivity may be associated with the subsequent risk of prostate cancer in men [43, 44] further alert researchers for possible associations of HPV-16 infection with other cancers with similar constellations of risk factors.

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